

WHAT IS CLAIMED IS:

1. A method of treating or identifying diseased tissues in a patient, comprising:

(A) administering to said patient a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

(B) optionally, administering to said patient a clearing composition, and allowing said composition to clear non-localized antibodies or antibody fragments from circulation;

(C) administering to said patient a first targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes; and

(D) when said targetable conjugate comprises an enzyme, further administering to said patient

1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or  
2) a drug which is capable of being detoxified in said patient to form an intermediate of lower toxicity, when said enzyme is capable of reconvertng said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

3) a prodrug which is activated in said patient through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of reconvertng said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific

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antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site.

2. The method of claim 1, further comprising, when said first targetable conjugate comprises a prodrug, administering a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and an enzyme capable of converting said prodrug to a drug or of reconverting a detoxified intermediate of said drug to a toxic form.

3. The method of claim 1, wherein said therapeutic agent comprises one or more radioactive isotopes useful for killing diseased tissue.

4. The method of claim 1, wherein said therapeutic agent comprises boron atoms, and said method further comprises the step of irradiating said boron atoms localized at said diseased tissue, thereby effecting BNCT of said diseased tissue.

5. The method of claim 4, wherein said boron atoms are boron-10 enriched.

6. The method of claim 1, wherein said therapeutic agent comprises one or more toxins.

7. The method of claim 1, wherein said therapeutic agent comprises one or more drugs.

8. The method of claim 1, wherein said therapeutic agent comprises one or more prodrugs.

9. The method of claim 1, wherein said diagnostic agent comprises one or more radioactive isotopes useful for detecting diseased tissue.

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10. The method of claim 1, wherein said diagnostic agent comprises one or more image enhancing agents for use in magnetic resonance imaging (MRI).

11. The method of claim 9, wherein said radioactive isotope is used to perform positron-emission tomography (PET).

12. The method of claim 1, wherein said at least one arm that specifically binds a targeted tissue is a monoclonal antibody or a fragment of a monoclonal antibody.

13. The method of claim 1, wherein said at least one other arm that specifically binds a targetable conjugate is a monoclonal antibody or a fragment of a monoclonal antibody.

14. The method of claim 1, wherein said at least one arm that specifically binds a targeted tissue is a humanized antibody or a fragment of a humanized antibody.

15. The method of claim 1, wherein said at least one other arm that specifically binds a targetable conjugate is a humanized antibody or a fragment of a humanized antibody.

16. The method of claim 1, wherein said targetable conjugate comprises a peptide.

17. The method of claim 1, wherein said targetable conjugate comprises a carbohydrate.

18. The method of claim 1, wherein said targetable conjugate comprises one or more haptens.

19. The method of claim 1, wherein said targetable conjugate comprises one or more chelators or metal-chelate complexes.

20. The method of claim 1, wherein said bi-specific antibody or antibody fragment further comprises a radionuclide.

21. The method of claim 16, wherein said peptide is X-Gly-D-Tyr-D-Trp-Gly-D-Lys(X)-Gly-D-Tyr-D-Trp-OH, where X represents a moiety selected from the group consisting of a free amino acid group, a protected amino acid group, a chelating agent and a metal-chelate complex.

22. The method of claim 16, wherein said peptide is Ac-Cys(Y)-D-Tyr-D-Trp-Gly-D-Cys(Y)-Gly-D-Tyr-D-Trp-OH, where Y represents a moiety selected from the group consisting of a free thiol group, a protected thiol group, a chelating agent and a metal-chelate complex.

23. The method of claim 16, wherein said peptide is Ac-Gly-D-iodo-Tyr-D-Trp-Gly-D-Lys(Ac)-Gly-D-iodo-Tyr-D-Trp-OH.

24. The method of claim 16, wherein said peptide comprises two or more different chelators which can be complexed selectively with different metal ions, one or both of said cations being a diagnostically useful radioisotope or a therapeutic radioisotope.

25. The method of claim 19, wherein at least one of said chelators is a hard base chelator for a hard acid cation, and at least one of said chelators is a soft base chelator for a soft acid cation.

26. The method of claim 25, wherein said hard base chelator comprises carboxylate and amine groups.

27. The method of claim 26, wherein said hard base chelator is DTPA, NOTA, DOTA or TETA.

28. The method of claim 19, wherein said soft base chelator comprises at least one sulfur-containing group.

29. The method of claim 28, wherein said soft base chelator is a substituted or unsubstituted thiosemicarbazonylglyoxylcysteine (TscGCys) or thiosemicarbazonylacetylcysteine (TscACys).

30. A kit useful for treating or identifying diseased tissues in a patient comprising:

(A) a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate;

(B) a first targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and one or more conjugated therapeutic or diagnostic agents, or enzymes; and

(C) optionally, a clearing composition useful for clearing non-localized antibodies and antibody fragments; and

(D) optionally, when said first targetable conjugate comprises an enzyme,

1) a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site; or

2) a drug which is capable of being detoxified in said patient to form an intermediate of lower toxicity, when said enzyme is capable of reconvertng said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

3) a prodrug which is activated in said patient through natural processes and is subject to detoxification by conversion to an intermediate of lower toxicity, when said enzyme is capable of

reconverting said detoxified intermediate to a toxic form, and, therefore, of increasing the toxicity of said drug at the target site, or

4) a second targetable conjugate which comprises a carrier portion which comprises or bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and a prodrug, when said enzyme is capable of converting said prodrug to a drug at the target site.

31. The kit of claim 30, further comprising, when said first targetable conjugate comprises a prodrug, a second targetable conjugate which comprises a carrier portion which comprises or <sup>carries?</sup> bears at least one epitope recognizable by said at least one other arm of said bi-specific antibody or antibody fragment, and an enzyme capable of converting said prodrug to a drug or of reconverting a detoxified intermediate of said drug to a toxic form.

32. A bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate.

33. The bi-specific antibody or antibody fragment of claim 32, wherein said antibody or antibody fragment is humanized.

34. The bi-specific antibody or antibody fragment of claim 32, wherein said antibody or antibody fragment is monoclonal.

35. A recombinant DNA construct comprising an expression cassette capable of producing in a host cell a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding said bi-specific

antibody or antibody fragment, and a transcriptional and translational termination regulatory region functional in said host cell, wherein said bi-specific antibody or antibody fragment is under the control of said regulatory regions.

36. A set of expression cassettes capable of producing a bi-specific antibody or antibody fragment having at least one arm that specifically binds to a targeted tissue and at least one other arm that specifically binds to a targetable conjugate, wherein each cassette comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in a host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a fragment of said bi-specific antibody, and a transcriptional and translational termination regulatory region functional in said host cell, wherein said fragment is under the control of said regulatory regions.

37. A method of preparing a bi-specific antibody or antibody fragment having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, comprising:

- (A) introducing the recombinant DNA construct of claim 1 into a host cell;
- (B) growing said cell and isolating said antibody or antibody fragment.

38. The method of claim 37, wherein said host cell is a mammalian cell.

39. The method of claim 38, wherein said mammalian cell is a zygote, and said introduction of said recombinant DNA construct produces a transgenic animal capable of producing said bi-specific antibody or antibody fragment.

40. The method of claim 37, wherein said host cell is an insect cell.

41. The method of claim 37, wherein said host cell is an bacterial cell.

42. The method of claim 37, wherein one or more arms of said bi-specific antibody or antibody fragment has multiple binding sites for its respective hapten.

43. A method of preparing a bi-specific fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, comprising:

- (1) (A) introducing into a host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said host cell, wherein said fragment of said bi-specific fusion protein is under the control of said regulatory regions;
- (B) co-introducing into said host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a light-chain antibody fragment which is complementary to said Fd fragment in (A) and which when associated with said Fd fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said host cell, wherein said light-



- chain antibody fragment is under the control of said regulatory regions;
- (C) growing said cell and isolating said bi-specific fusion protein, or
- (2) (A) introducing into a first host cell a recombinant DNA construct comprising an expression cassette capable of producing in said first host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first host cell, a translational initiation regulatory region functional in said first host cell, a DNA sequence encoding a scFv linked to a Fd fragment, and a transcriptional and translational termination regulatory region functional in said first host cell, wherein said fragment of said bi-specific fusion protein is under the control of said regulatory regions;
- (B) introducing into a second host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second host cell a light-chain antibody fragment which is complementary to said Fd fragment in (2)(A) and which when associated with said Fd fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second host cell, a translational initiation regulatory region functional in said second host cell, a DNA sequence encoding a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said second host cell, wherein said light-chain antibody fragment is under the control of said regulatory regions;
- (C) growing said first and second host cells;
- (D) optionally isolating said bi-specific fusion protein fragment and said light-chain antibody fragment; and

(E) combining said fragments to produce a bi-specific fusion protein and isolating said bi-specific fusion protein.

44. A method of preparing a bi-specific fusion protein having at least one arm that specifically binds a targeted tissue and at least one other arm that specifically binds a targetable conjugate, comprising:

- (1) (A) introducing into a host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said host cell, wherein said fragment of said bi-specific fusion protein is under the control of said regulatory regions;
- (B) co-introducing into said host cell a recombinant DNA construct comprising an expression cassette capable of producing in said host cell a Fd fragment which is complementary to said light-chain antibody fragment in (A) and which when associated with said light-chain antibody fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said host cell, a translational initiation regulatory region functional in said host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said host cell, wherein said Fd fragment is under the control of said regulatory regions;
- (C) growing said cell and isolating said bi-specific fusion protein, or
- (2) (A) introducing into a first host cell a recombinant DNA

construct comprising an expression cassette capable of producing in said first host cell a fragment of said bi-specific fusion protein, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said first host cell, a translational initiation regulatory region functional in said first host cell, a DNA sequence encoding a scFv linked to a light-chain antibody fragment, and a transcriptional and translational termination regulatory region functional in said first host cell, wherein said fragment of said bi-specific fusion protein is under the control of said regulatory regions;

(B) introducing into a second host cell a recombinant DNA construct comprising an expression cassette capable of producing in said second host cell a Fd fragment which is complementary to said light-chain antibody fragment in (2)(A) and which when associated with said light-chain antibody fragment forms a Fab fragment whose binding site is specific for said targeted tissue, wherein said construct comprises, in the 5' to 3' direction of transcription, a transcriptional initiation regulatory region functional in said second host cell, a translational initiation regulatory region functional in said second host cell, a DNA sequence encoding a Fd fragment, and a transcriptional and translational termination regulatory region functional in said second host cell, wherein said Fd fragment is under the control of said regulatory regions;

(C) growing said first and second host cells;

(D) optionally isolating said bi-specific fusion protein fragment and said Fd fragment; and

(E) combining said fragments to produce a bi-specific fusion protein and isolating said bi-specific fusion protein.

*uses two  
host cells*

45. The method of claim 43, wherein said host cell is a mammalian cell.

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46. The method of claim 43, wherein said host cell is an insect cell.
47. The method of claim 43, wherein said host cell is a bacterial cell.
48. The method of claim 44, wherein said host cell is a mammalian cell.
49. The method of claim 44, wherein said host cell is an insect cell.
50. The method of claim 44, wherein said host cell is a bacterial cell.

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